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Persistence of azoxystrobin in cumin crop cultivated on sandy loam soils of Rajasthan, India

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Abstract

Cumin (*Cuminum cyminum* L.) belonging to *Apiaceae* family is intensively cultivated in India, China, Japan, Indonesia and Mediterranean countries. Cumin herb is sensitive to humidity resulting into disease pests and needs protection many a times against fungal diseases and insect pest infestation. The common fungal disease like wilt is caused by *Fusarium* sp. and blight by *Alternaria burnsii*, infestation due to sucking pests such as aphids and thrips are the common issues. These diseases reduce the crop yield drastically if not protected with agro-chemicals. Persistence of azoxystrobin was studied in cumin when applied @ 115 g ai ha^{-1} (recommended dose) and 230 g ai ha^{-1} (double the recommended dose. A total of three sprays were given at an interval of 15 days. Cumin and soil samples were collected after 3rd spray, extracted and analysed by gas chromatography using electron capture detector. Half life of azoxystrobin on cumin plant varied from 7.0 to 9.1 days. Residues of azoxystrobin were much below the prescribed MRL (0.05 mg kg^{-1}) after 36 days at harvest. The dissipation of azoxystrobin in soil followed first order rate kinetics with an average half life of 8.1 days at the recommended dose of application.

Key words : Azoxystrobin, Cumin plant, Persistence, Soil

Introduction

Cumin (*Cuminum cyminum* L.) a member of the Apiaceae has been widely used as an organoleptic nutritional ingredient in food cuisines and savouries. It is intensively cultivated in India, China, Morocco, Japan, Indonesia, Algeria & Turkey (Tuncturk & Tuncturk, 2006). In India cumin is prominently grown in the arid and semi-arid regions of Rajasthan and Gujarat which have favourable climatic and soil conditions for its cultivation. Cumin accounts for 20-30% of total Indian spices exports. Apart from cumin, the by-products of cumin seeds which is oleoresin and cumin oil is also exported from India. Cumin herb is sensitive to humidity and frost, has many enemies in terms of fungal diseases and insect pest infestation. These diseases reduce the crop yield up to >70% if not protected with agro-chemicals.

In India apart from commonly used alkylene bis dithiocarbamates some other fungicides are also being used for protection from fungal diseases in cumin. Some of the non systemic, systemic and broad spectrum molecules like chlorothanil, azoxystrobin, propiconazole and difenconazole are being used by farmers on cumin crop. The dissipation rate, efficacy, half life and the pre-harvest intervals (PHIs) for azoxystrobin fungicide is being evaluated in this valuable export commodity. The information on the residue retention and dissipation of azoxystrobin in cumin plant, seed and soil under cultivation in arid and semi-arid agro climatic conditions as well as harvest time residue in soil and cumin seeds is not available. Pesticide dissipation on crops is dependent on the environmental conditions, besides the crop species and stage of application, therefore, their dissipation pattern may vary under different agro-climatic conditions.

Azoystrobin is a new strobilurin fungicide that is relatively non-toxic to humans and the environment, apart from dangers to aquatic species and ground waters. However, in spite of the very short time since its introduction and widespread use resistance and cross-resistance are now common in two continents (Clough and Godfrey, 1996).

In order to ensure consumer safety, European Union (http://ec.europa.eu/sanco_pesticides/public/index.cfm) has set up Maximum Residue Limit (MRL). These MRL value for azoystrobin (0.05 mg kg $^{-1}$), have been set by EU respectively in cumin seeds. MRLs set at such low levels require a sensitive and robust analytical method. Therefore it is important to ensure that the levels of residue of the azoystrobin at the time of harvest in the food stuffs do not pose any hazard to the consumers and its level should be below the acceptable limits in domestic as well as in international markets. The pre-harvest interval (PHI) was evaluated on the basis of the dissipation pattern of this chemical.

This study was conducted to evaluate the residue dynamics of azoystrobin on cumin and soil during croping season i.e. November-March 2015-2016 and ensuring the

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scientific application of these formulation in cumin ecosystem for safe use for human health as well as environment. The analytical method used for analysis of azoystrobin fungicides in cumin seeds and soil was validated as per method validation guidelines (Method validation and quality control procedures for pesticide residues analysis in food and feed, SANCO/12495/2011).

Materials and methods

Chemicals and Reagents

Reference standards of azoystrobin (purity 95 %) were procured from Sigma Aldrich, Germany. Primary secondary amine (PSA), particle size 40 µm, was procured from Agilent Technologies, India. Acetonitrile and water used were of HPLC- grade and procured from Merck Chemicals, Mumbai, India. Hexane, acetone and anhydrous magnesium sulphate were of analytical grade (AR) and procured from SRL, Pvt. Ltd. Mumbai, India. Anhydrous sodium sulfate was procured from Thomas Baker, Bangalore. It was activated in an oven at 110 °C for 5 h and kept in desiccators before use. Stock solutions containing 1000 µg mL^{-1} of azoystrobin was prepared by dissolving 10 (± 0.1) mg reference standards in 10 ml of HPLC-grade acetonitrile and stored at -4 ^{0}C. The required dilutions of known concentrations (100.0, 10.0, 5.0, 0.1, 0.05 and 0.01 µg mL^{-1}) were prepared in HPLC-grade acetonitrile by serial dilution of stock solution.

Field experiment

Field experiment was conducted during the croping seasion at the ICAR-National Research Centre on Seed Spices Experimental farm (Tabiji, Ajmer, India; longitude: 74^{0}35'39" E and latitude 26^{0}22'12"N; 460.17 MSL) as per standard procedures. A randomised block design was used with three replications for each treatment and plots (4.0 x 3.0 m^2) were separated by ridges. The cumin variety GC-4 was grown. Azoystrobin was applied to the cumin plants at recommended dosage of 115gm ha^{-1}. and 230 gm ha^{-1} for single dose and double dose respectively. The first 1st, 2nd and 3rd foliar sprays of azoystrobin were given at 60, 75 and 90 days after sowing (DAS).

Sampling

Cumin plant samples were randomly collected from each replicate of the treated and control plots separately at 0 (within 4 h after third spraying) 1, 3, 5, 7, 9 and 15 days after the final spray and finally at crop harvest time. Five soil samples (0-15 cm depth) were collected at crop harvest time. The soil samples were processed following standard protocol. The cumin seeds were separated from the plants and stored at -20 ^{0}C for analysis.

Sample extraction and cleanup

Cumin contains ~ 10 % of moisture therefore addition of water is essential to get thorough homogenisation. Also to increase the extractability of incurred residues, water addition in dry commodity was recommended in SANCO guideline. The samples were crushed thoroughly in a mixer and grinder after adding HPLC grade water (1:1 sample: water) and 25 g of crushed sample was weighed in a conical flask and then fortified further at 0.05 µg g^{-1} concentration level using standard solution of azoystrobin. Several methods have been reported in literature for pesticide residue analysis in various fruits and vegetables using acetonitrile (Anastassiades & Lehotay, 2003; Lehotay, Mastovska, & Lightfield, 2005, Dubey *et al*., 2016) and ethyl acetate (Mol *et al*., 2007) as extraction solvents and further cleanup of the samples using dispersive solid phase extraction (dSPE). The powdered cumin sample (2 g) was pre-soaked in distilled water (10 ml) for 30 min. The extraction was done with acetonitrile (10 ml) in presence of anhydrous magnesium sulfate (4 g) and sodium chloride (1 g) by means of vortexing (2 min), followed by centrifugation at 5000 rpm for 5 min. d-SPE cleanup with 75 mg PSA and 100 mg C18 was given to 1 ml acetonitrile extract. Fifty gram of soil sample was also extracted by 150 mL of acetonitrile thrice with the similar procedure without any further cleanup. The cleaned extract was analyzed by GC-MS/MS.

Validation Study

Analytical method validation was performed according to the DG SANCO guidelines (DG SANCO Document No. SANCO/12571/2013) as mentioned below.

Selectivity and sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were decided as the smallest measured quantity in cumin matrix at which the signal to noise ratio (S/N) were 3:1 and 10:1, respectively. At LOQ the quantifier MRM has an S/N above 3:1.

Accuracy and precision

Accuracy and precision of the analytical method was performed using blank samples of untreated soil, cumin plant and seeds. Linearity was performed by analysing solvent blank spiked at seven concentration levels between 0.05 and 5 µg mL^{-1}. The method was assessed with recovery experiments at three spiking concentrations (0.1, 0.5 and 1 µg g^{-1}) in five replicates to determine the accuracy and precision of the method. Precision was evaluated in terms of repeatability and reproducibility. The matrix effect was evaluated by post-extraction spiking at 5, 10 and 15 ng g^{-1} levels and in comparison to the response of the solvent standards. Matrix match calibrations of respective matrices were used for calculation of recovery (%). The repeatability of the method (precision) was estimated in terms of % RSD (n=3).

Data analysis

The residue data were subjected to first order kinetics ($\log C_t = \log C_0 - K_t /2.303$), where C_t is the concentration (μg g^{-1}) after a lapse of time (t); C_0 is the apparent initial concentration (μg g^{-1}) and K is the dissipation constant. The value of K was obtained from the slope value (K= 2.303 x slope) while the half life was calculate using the equation $T_{1/2} = 0.693/K$.

Results and discussion

The studies on dissipation rate constant (K), regression equation, correlation coefficient (R^2) and half-life values of azoxystrobin pesticides in cumin plant, soil and cumin seeds with respect to single and double dose treatments at 0 (within 4 h after third spraying) 1, 3, 5, 7, 9, 15, 30 days and at harvest (36 day) are presented below in table 1. Status of azoxystrobin was detected on 0 day itself and its residue showed gradual decrease thereafter with passage of time. Its concentration goes below the detection limits in cumin plant samples at harvest in single dose and to 0.13 ppm in cumin plant samples treated with double dose of the pesticide under study. Similarly in soil were the crop was sown the concentration goes below the detection limits both in single and double doses. In harvested cumin seeds the residue concentration of azoxystrobin goes below the detection limits in single dose and it was recorded to be 0.004 ppm in double dose treatment (Utture *et al.,* 2011). The regression equation, correlation coefficient (r^2) and dissipation rate constant (k) respectively has been presented in the table. 1. The disappearance curves for azoxystrobin in cumin field soil are presented in fig. 1.

The degradation kinetics of azoxystrobin in cumin seeds

Table 1. Regression equation, Ccorrelation coefficient (R^2), dissipation rate constant (K), half-life and harvest time residue

Matrix	LOQ (mg/kg)	Pesticide application rate	Regression equation	Correlation coefficient (R^2)	K	Half-life (days)	HTR* (mg/kg)
Cumin Plant	0.08	SD*	y = -0.045x - 0.256	0.961	0.103	7	BDL
		DD *	y = -0.033x + 0.127	0.980	0.076	9.1	0.13
Soil	0.05	SD	y = -0.055x - 0.388	0.991	0.126	5	BDL
		DD	y = -0.048x - 0.125	0.983	0.11	6.3	BDL
Cumin Seeds	0.08	SD	-	-	-	-	BDL
		DD	-	-	-	-	0.004

HTR-Harvest time residues; SD-single dose; DD- double dose and BDL-below detection level.

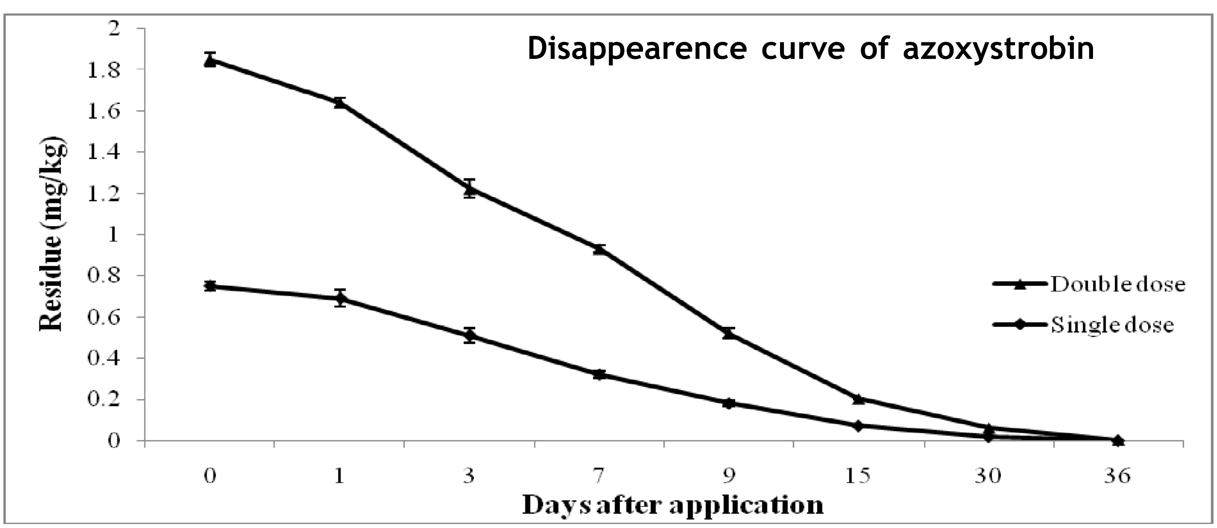

Fig. 1: The disappearance curves for azoxystrobin in cumin field soil

is presented in table 2. The data shows that the dissipation proceeds gradually and it diminishes to negligible level almost below detection limit after 36 days of harvest.

Similar study by Lentzarizos *et al.*, 2006; Sendhilvel *et al.*, 2004, on residues of azoxystrobin from grapes to raisins suggest the safety of azoxystrobin being used in these commodity, it degrades rapidly under agriculture field conditions with a soil half life of less than two weeks. The compound is non-volatile and does not leach, photolysis accounts for the majority of the initial loss of the compound, the remainder being degraded microbially. Clough and Godfrey, 1996 evaluated plant metabolism in three diverse crops--grapes, wheat and peanuts--which should serve to define the metabolism of azoxystrobin in a wide range of crops. Azoxystrobin does not accumulate in crop seeds or fruits; very low residues are found in wheat grain, banana pulp and peanut. Metabolism of azoxystrobin in plants is complex with more than 15 metabolites identified. These metabolites are present at low levels, typically much less than 5 percent of the Total Recoverable Residue (TRR).

Table. 2. Degradation kinetics of azoxystrobin in cumin seeds.

Single dose (115 ppm/ha)					Double dose (230 ppm/ha)				
C	**ppm**	**Log Ct**	**Residue disappearance**	**% dissipation**	**C**	**ppm**	**Log Ct**	**Residue disappearance**	**% dissipation**
0	0.41	-0.387	0	-	0	0.79	-0.102	0.139	-
1	0.35	-0.456	0.146	14.63	1	0.68	-0.167	0.139	13.92
3	0.28	-0.553	0.317	31.71	3	0.51	-0.292	0.354	35.44
5	0.21	-0.678	0.488	48.79	5	0.42	-0.377	0.292	29.23
7	0.19	-0.722	0.536	53.66	7	0.37	-0.432	0.332	33.18
9	0.12	-0.921	0.707	70.736	9	0.24	-0.620	0.696	69.62
15	0.06	-1.222	0.854	85.37	15	0.15	-0.824	0.810	81.01
At harvest ï Below Detection Level					At harvest - Below Detection Level				

Conclusion

The study suggests that when azoxystrobin was applied for blight protection at the recommended and double the recommended rate of application, no residues were detected at harvest in cumin. Thus, the application of azoxystrobin was found to be safe from the consumer's point of view as well as for export purposes.

References

Anastassiades, M., and Lehotay, S. J. 2003. Fast and easy multiresidue method employing ecetonitrile extraction/portioning and "dispersive solid phase extraction" for the determination of pesticide residues in produce. *J. AOAC International*, 86, 412-431.

Clough, J. M. and Godfrey, C. R. A. 1996. Azoxystrobin: A Novel Broad-spectrum Systemic Fungicide, *Pesticide Outlook*, 7:16-20.

http://www.eurlpesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf

Dubey, P.N., Kant, K., Ahammed Shabbir, T.P., Saxena, S.N., Rathore, S.S. and Diwakar, Y. 2016. Multi residuse evaluation protocol for coriander (*coriander sativum*) seeds. *J. Seed spices*, 6(1) : 82-85.

Lehotay, S. J., Mastovska, K., and Lightfield, A. R. 2005. Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC International*, 88,615-629.

Lentzarizos, C., Avramides, E. J., Kokkinaki, K. 2006. Residues of azoxystrobin from grapes to raisins. *J Agric Food Chem*. 2006;54:138–141.

Mol, H. G. J., Rooseboom, A., Vam Dam, R., Roding, M., Arondeus, K. and Sunarto, S. 2007. Modification and re-validation of the ethyl acetate-based multi residue method for pesticide in produce. *Analytical Bioanalytical Chemistry*, 389, 1715-1754.

SANCO 2011. Method validation and quality control procedures for pesticide residues analysis in food and feed, Document No SANCO/12495/2011.

Sendhilvel, V., Raghuchander, T., Nakkeran, S., Amutha, G., Marimuthu, T. 2004. Bioefficacy of azoxystrobin against downy mildew of grapewine. *Pestology*. 2004; 18:44–51.

Tuncturk, R., Tuncturk, M. 2006. Effects of different phosphorus levels on yield and quality components of cumin (*Cuminum cyminum* L.), *Res. J.Agric. Biol. Sci.*, 2, 336-340.

Utture, S. C., Banerjee, K., Dasgupta, S., Pati, S. H., Jadhav, M. R., Wagh, S. S., Kolekar, S. S., Anuse, M. A., Adsule, P. G. 2011. Dissipation and distribution behavior of azoxystrobin, carbendazim, and difenoconazole in pomegranate fruits. *J Agric Food Chem* 59:7866–7873.

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